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13. ABSTRACT ***(Maximum 200 Words)***

Estrogen receptor α (ERα) negative breast tumors often overexpress growth factor receptors such as EGFR or c-erbB2 as compared to their ERα+ counterparts. This results in increased growth factor signaling and leads to hyperactivation of MAPK (ERK 1 and ERK 2) in these ERα-negative cells. We have previously shown that the overexpression of growth factor signaling components in ERα+ MCF7 cells results in MAPK hyperactivation and ERα downregulation without inducing its transcriptional activation. ERα downregulation in these cells is a specific action of MAPK hyperactivation that can be reversed in the presence of dominant negative ERKs or inhibitors of MAPK activation. To determine the mechanism of this ERα downregulation, we assessed whether specific hyperactivation of ERK1 or ERK2 was responsible. ERα downregulation is not an ERK-1 or -2 specific effect, as expression of either dnERK or both returns ERα expression in these cells. TAM67, a dominant negative jun construct, prevents AP-1 transcriptional activity, and was also transiently transfected into model cell lines. AP-1 activity increases in response to MAPK activation, and high AP-1 activity has been observed in ERα-negative/hormone independent breast cancers. However, TAM67 did not reduce ERα expression in our ERα-negative cells, indicating mechanistically that while there is correlative data between increasing AP-1 activity and hormone independence/ERα-negativity, increased AP-1 activity does not directly result in ERα downregulation.

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Table of Contents

Cover 1

SF 298 2

Introduction 4

Body 4-5

Key Research Accomplishments 6

Reportable Outcomes 6

Conclusions 6

References.....7

Appendices 8-10

Introduction

Upon diagnosis, breast cancer is described as either estrogen receptor (ER)-positive or ER-negative. Patients with ER-positive tumors have a longer disease free and overall survival, and they respond better to hormonal therapies such as tamoxifen, which is easier to tolerate than cytotoxic chemotherapy [1]. Conversely, patients with ER-negative tumors tend to have more aggressive disease and must be relegated to much harsher chemotherapy regimens [2;3]. Unlike ER-positive tumors, ER-negative tumors tend to overexpress growth factor receptors such as EGFR and c-erbB-2, and they have been shown to have high levels of activation of downstream signaling molecules such as MAPK [4-6]. Previous studies indicated that the hyperactivation of MAPK is directly responsible for the downregulation of ER in breast cancer cells, and that this downregulation is reversible via abrogation of MAPK activity [7]. Consequently, the present study seeks to identify the mechanism of this MAPK induced phenomenon. The outcome of this study has the potential to impact the lives of breast cancer patients who may be able to benefit from a treatment protocol where the blocking of growth factor signaling through MAPK can return ER expression and tamoxifen sensitivity, allowing ER-negative patients to avoid the harsh side effects of cytotoxic chemotherapy.

Body

Statement of Work

Task 1. Identify whether MAPK-induced downregulation of ERα is mediated specifically by ERK1 or ERK2. (months 1-8)

- *Overexpress ERK1 or ERK2 using activated, wild type ERK constructs*

Wild type ERK constructs were obtained from Melanie Cobb. While data from Dr. Cobb's lab indicated that these wild type constructs were fully activated by serum (personal communication), when these constructs were overexpressed in ER-positive MCF7 breast cancer cells in the presence of serum, they did not appear to be active. Phospho-MAPK western blots, as well as western blots for downstream effectors activated by active MAPK, showed no increase in activity with the expression of these constructs. Therefore, abrogation with dominant negative constructs would be the most informative experiment to determine impact of ERK signaling.

- *Abrogate ERK1 and ERK2 mediated signaling via dominant negative ERK1 and ERK2 constructs*

Overexpression of both dominant negative ERK 1 and dominant negative ERK2 together resulted in the return of ER at the highest level in all three ER-negative cell lines, while overexpression of either construct alone was also sufficient to return ER activity (Figure 1). Therefore, to the extent that can be presently assessed, the downregulation of ER by high MAPK activity does not appear to be mediated specifically by either ERK.

Task 2. Identify the role of AP-1 and its composition in ERα downregulation. (months 6-18)

- *Determine AP-1 composition in ERα-negative and ERα+ cell lines using fos and jun family member-specific antibodies by Western blotting and antibody supershifting*

Pending final results of the second part outlined in Task 2, this section has not been completed. As preliminary data indicate that abrogation of AP-1 activity does not play a role in the downregulation of ER in these model cell lines, this set of experiments may be omitted. However, Santa Cruz makes a series of antibodies against all fos and jun family members that can be easily obtained should the need arise.

- *Abrogate AP-1 expression using a dominant negative jun construct, Tam67*

The Tam67 construct was obtained from Powell Brown, and is used to abrogate all AP-1 driven transcription. Overexpression of this construct in the ER-negative model cell lines does not result in the reversal of ER downregulation (Figure 2). Therefore, while there is significant clinical data correlating high levels of AP-1 activity with ER-negativity and hormone independence [8-10], this is the first data to demonstrate that high levels of AP-1 activity do not directly result in the downregulation of ER in breast cancer.

Task 3. Assess the role of $pp90^{RSK}$ in MAPK-induced ERα repression. (months 18-36)

- *Compare $pp90^{RSK}$ activity levels in ERα-negative and ERα+ cell lines using ant anti-phospho-$pp90^{RSK}$ antibody*

We are in possession of the Cell Signaling antibody that is raised against the activating phosphorylation site of pp90RSK. These experiments can be rapidly performed after the appropriate cellular lysates have been made.

- *Generation of a constitutively active $pp90^{RSK}$ construct*

A constitutively active RSK construct has been obtained from Dr. Jeffrey Smith, and a dominant negative RSK construct has been obtained from Dr. John Blenis, so the construction of any constructs will not be necessary for the completion of this task.

- *Determine if $pp90^{RSK}$ overexpression causes ERα downregulation in ERα+ cell lines*

Again, the recently obtained constitutively active construct will be used in transient transfection luciferase reporter assays in ER-positive cells to accomplish this goal. In addition, use of the dominant negative construct in ER-negative cells will indicate whether the abrogation of signaling through RSK can result in the re-expression of ER.

Key Research Accomplishments

- Determination that the abrogation of either ERK1 or ERK2 or both ERKs leads to the reversal of ER downregulation
- Determination that abrogation of AP-1 mediated transcription does not reverse ER downregulation in ER-negative model cell lines
- Obtained ERK2Δ19-25 construct [11] which can be used to determine whether a nuclear or cytoplasmic substrate of MAPK is responsible for the downregulation of ER
- Obtained key reagents for the completion of project

Reportable Outcomes

Abstracts

Holloway, J.N., Alexander, J., and El-Ashry, D. A Substrate of MAPK is Responsible for the Downregulation of ERα in Breast Cancer Cells. 93rd Annual Meeting of the American Association for Cancer Research, San Francisco, CA, 2002. Abstract # 5332.

Conclusions

Previous data from our lab indicated that hyperactivation of MAPK results in the downregulation of ER in ER-positive breast cancer cells, and that this downregulation is reversible through the abrogation of both ERK1 and ERK2, either through MEK inhibition with U0126, or through the use of dominant negative constructs. We have now demonstrated that this ER downregulation is not a result of a specific substrate of either ERK1 or ERK2, as abrogation of either ERK or a combination of the two will result in the return of ER in ER-negative cells. As AP-1 family members are key MAPK substrates, we examined the effect of AP-1 abrogation on ER-negative cell lines to determine if clinical data correlating high AP-1 activity with ER-negativity had a causative relationship. Our data indicate that high AP-1 activity does not result in the downregulation of ER in our model cell lines, and this is the first data demonstrating that while there is significant clinical data correlating ER-negativity with high AP-1 activity, this AP-1 activity is not responsible for the downregulation of ER and acquisition of hormone independence. Future experiments aim to determine whether the activity of RSK, a key cytoplasmic substrate of MAPK, is involved in the loss of ER in breast cancer cells. Should RSK also not play a role, then the ERK2Δ19-25 construct will enable us to determine whether the key substrate resides in the cytoplasm or the nucleus. Determining the identity of the MAPK substrate that is responsible for ER downregulation may enable ER-negative patients to be treated with an inhibitor of that specific molecule, returning ER expression and tamoxifen sensitivity, allowing them to be treated with hormonal therapy and forgo the side effects that accompany cytotoxic chemotherapy.

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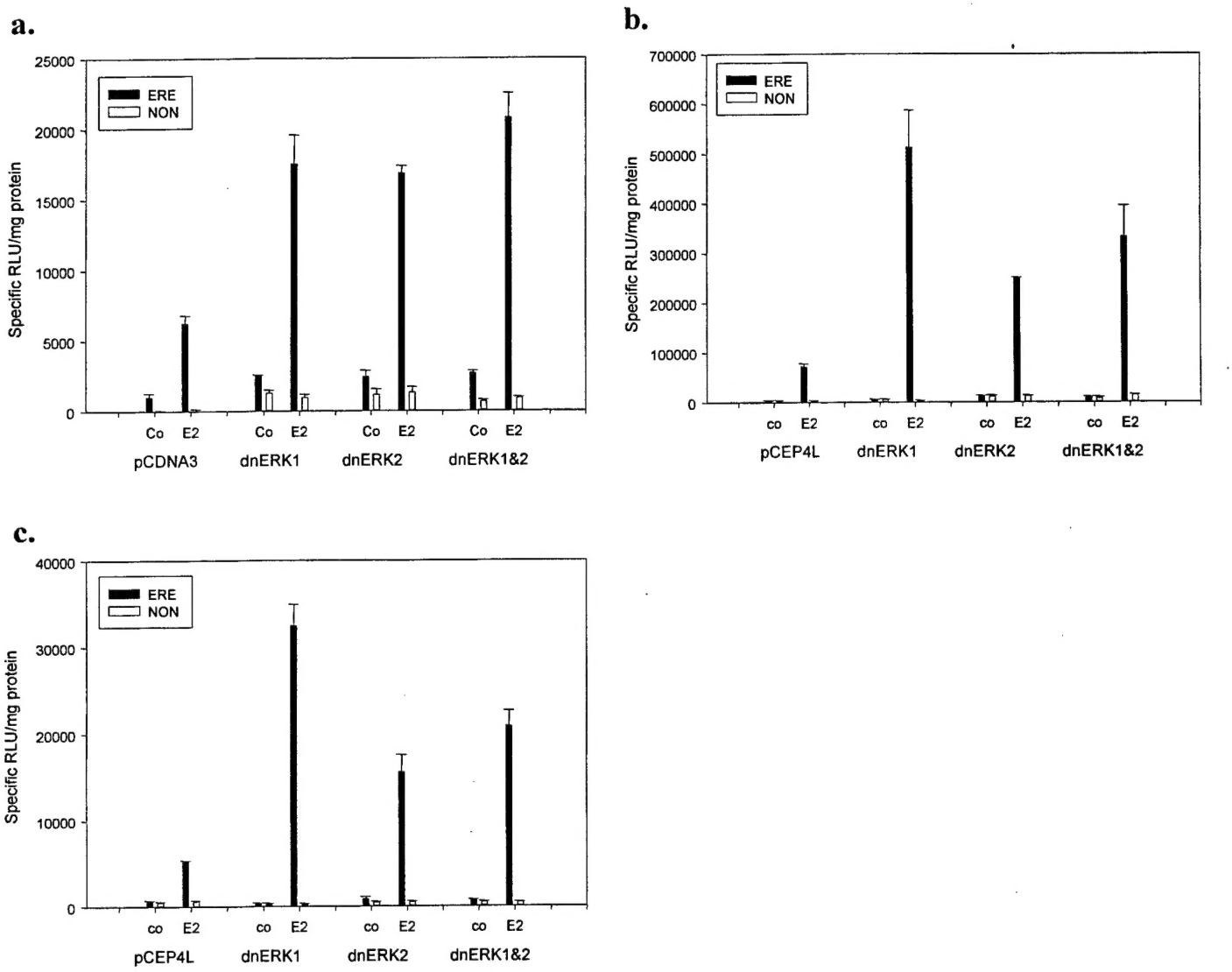
Appendices

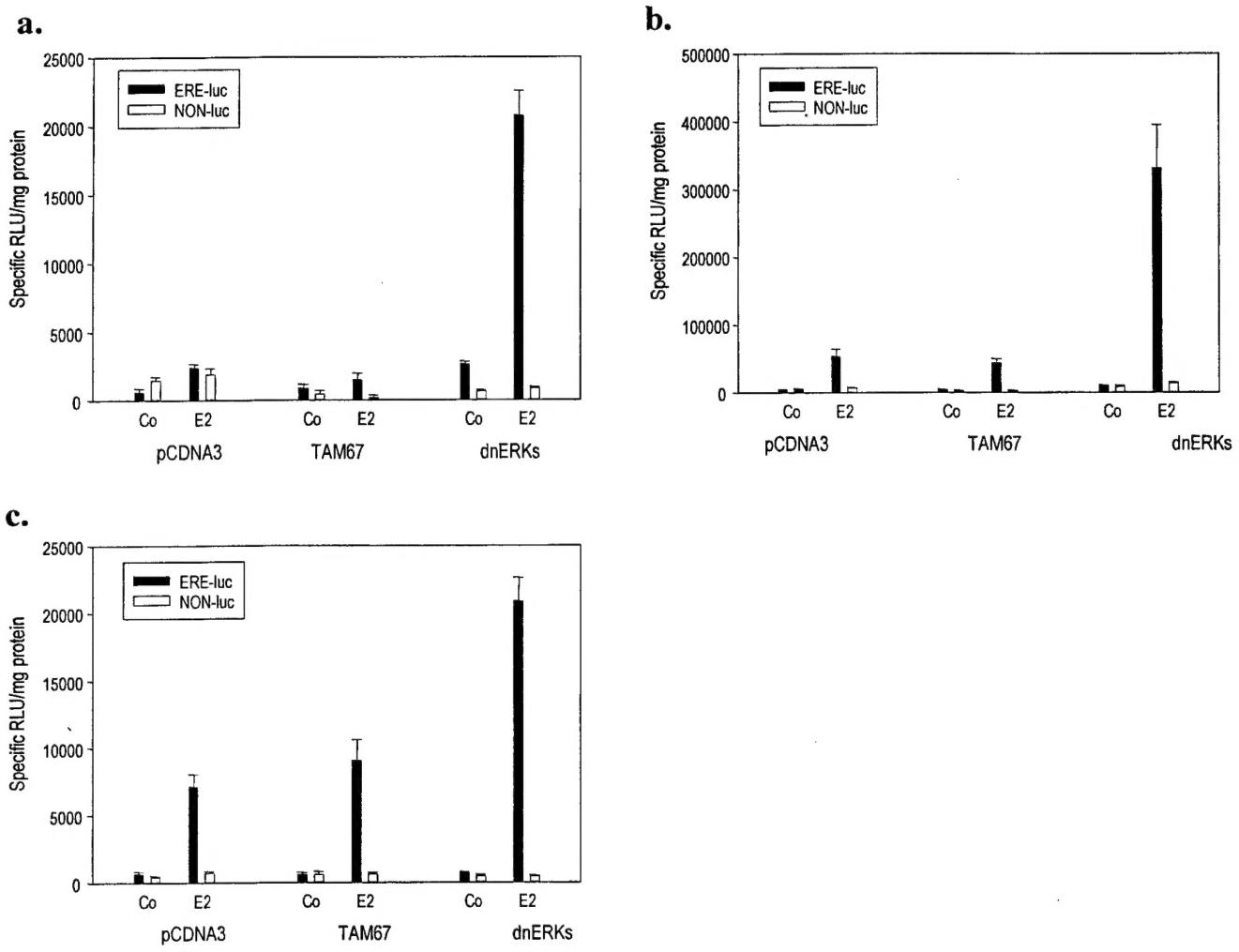
Figures 1 and 2 follow.

Figure 1.



Downregulation of ERα is not mediated exclusively by either ERK1 or ERK2. a.)Raf14c, b.)Mek15c and c.) MB3 cells were transiently co-transfected with 1.25 mg total dominant negative ERK constructs and 0.75 mg luciferase reporter constructs in triplicate in 6-well plates. ERE-luciferase was measured as an indication of ERα activity, which previous data has indicated correlates with ERα expression. NON-luciferase is a control with a scrambled ERE sequence to show the activation of the promoter driving the construct.

Figure 2.



Downregulation of ERα is not mediated by AP-1 activity. a.)Raf14c, b.)Mek15c and c.) MB3 cells were transiently co-transfected with 1.25 mg TAM67, a dominant negative jun construct, and 0.75 mg luciferase reporter constructs in triplicate in 6-well plates. ERE-luciferase was measured as an indication of ERα activity, which previous data has indicated correlates with ERα expression. NON-luciferase is a control with a scrambled ERE sequence to show the activation of the promoter driving the construct.